EPA

Attachment 2

Systematic Review of Chloroprene [CASRN 126-99-8] Studies Published Since 2010 IRIS Assessment to Support Consideration of the Denka Request for Correction (RFC)

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Integrated Risk Information System
National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency

DISCLAIMER

This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication.

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List of Abbreviations

ADME	absorption, distribution, metabolism, excretion
BMD	benchmark dose
BMI	body mass index
BW3/4	body-weight scaling to the 3/4 power
C1-MA-I	4-chloro-3-oxobutyl mercapturic acid
C1-MA-II	4-chloro-3-hydroxybutyl mercapturic acid
C1-MA-III	3-chloro-2-hydroxy-3-butenyl mercapturic acid
CASRN	Chemical Abstracts Service registry number
CD	β-chloroprene
CEO	(1-chloroethenyl)oxirane
DHBMA	3,4-dihydroxybutyl mercapturic acid
EPA	U.S. Environmental Protection Agency
HAWC	Health Assessment Workspace Collaborative
HERO	Health and Environmental Research Online
HOBMA	4-hydroxy-3-oxobutyl mercapturic acid
IISRP	International Institute of Synthetic Rubber Producers
IRIS	Integrated Risk Information System
IUR	inhalation unit risk
Km	Michaelis-Menten constant
MA	mercapturic acid
MCMC	Markov-Chain Monte-Carlo
MHBMA	2-hydroxy-3-butenyl mercapturic acid
MOA	mode of action
NATA	National Air Toxics Assessment
PBPK	physiologically based pharmacokinetic
PECO	population, exposure, comparator, and outcome
PK	pharmacokinetic
PBPK	physiologically based pharmacokinetic
PKWG	Pharmacokinetic Working Group
RD	respiratory depression
RFC	Request for Correction
RfC	reference concentration
ROBINS-I	Risk of Bias in Nonrandomized Studies of interventions
SMR	standardized mortality ratio
Vmax	maximum expiratory flow

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1. BACKGROUND

The U.S. Environmental Protection Agency (EPA) completed the most recent Integrated Risk Information System (IRIS) assessment of chloroprene in 2010. In that assessment, the agency concluded that chloroprene is "likely to be carcinogenic to humans" through a mutagenic mode of action (MOA) and that the primary exposure route of concern is the inhalation pathway. Accordingly, the assessment included an inhalation unit risk (IUR), which is an estimate of inhaled cancer potency that can be used to estimate the risk of cancer that would be expected in a population exposed to chloroprene in the air every day over a lifetime.

In 2015, the Office of Air and Radiation released the most recent version of the National Air Toxics Assessment (NATA), a national analysis that combines information about the emissions of specific air pollutants to estimate the risk of developing a particular health effect in a population. This NATA was the first to incorporate information (i.e., the IUR) from the 2010 IRIS assessment for chloroprene, and it identified the census tract in the vicinity of the Denka Performance Elastomers (Denka) facility in La Place, LA (i.e., Lake Pontchartrain Works site) as having an elevated risk for cancer.

In response to this designation on August 9, 2016, scientists from Ramboll Environ, as representatives of Denka briefed Agency scientists on specific issues related to the chloroprene assessment and new studies published since the release of the 2010 IRIS assessment. The conclusion of the Ramboll Environ scientists was that their new analyses provided a sufficient reason for IRIS to re-evaluate the science surrounding chloroprene and to update the IRIS assessment and derive new risk values. Subsequently, on June 26, 2017, a Request for Correction (RFC) was received by EPA from Robert Holden, Attorney for Denka Performance Elastomer LLC.

The purpose of this systematic review is to provide information on EPA's evaluation of the recent studies identified by Ramboll Environ scientists as well as other studies published since the 2010 IRIS assessment. This information will be considered as part of developing the EPA response to specific statements in the RFC.

2. OVERALL OBJECTIVES, SPECIFIC AIMS, AND POPULATION, EXPOSURE, COMPARATOR, AND OUTCOME (PECO) FRAMEWORK

The overall objective of this systematic review is to identify and evaluate human health-related studies of chloroprene published since the 2010 IRIS assessment to determine whether any new evidence is likely to have an impact on the current IRIS toxicity values (2×10^{-2} mg/m^3 reference concentration [RfC] or 3×10^{-4} mg/m^3 IUR).

2.1. SPECIFIC AIMS

- Identify literature pertaining to the health hazards of chloroprene as outlined in the population, exposure, comparator, and outcome (PECO) framework.

- Conduct study evaluation (risk of bias and sensitivity) for individual epidemiological and animal toxicity studies.

- Conduct study evaluation (reporting quality and applicability) for individual (physiologically based pharmacokinetic [PBPK], absorption, distribution, metabolism, excretion [ADME]) studies and any mechanistic studies prioritized according to the PECO framework.

- Summarize findings and assess whether any new evidence is likely to have an impact on the current IRIS toxicity values (2×10^{-2} mg/m^3 RfC or 3×10^{-4} mg/m^3 IUR).

2.2. POPULATION, EXPOSURE, COMPARATOR, AND OUTCOME (PECO) FRAMEWORK

A PECO framework (see Table 1) is used as an aid to focus the research question(s), search terms, and inclusion/exclusion criteria in a systematic review.

Table 1. Population, exposure, comparator, and outcome (PECO) framework

PECO Element	Evidence
Population	**Human:** Any population (occupational, general population, including children and other sensitive population). The following study designs will be considered most informative: controlled exposure, cohort, case-control, or cross-sectional. Note: Case reports and case series will be tracked during study screening but are not the primary focus of this assessment.
	Animal: Nonhuman mammalian animal species (whole organism) of any life stage (including preconception, in utero, lactation, peripubertal, and adult stages).
	Nonmammalian model systems/in vitro/in silico: Nonmammalian model systems such as fish, amphibians, birds, invertebrates, e.g., *Caenorhabditis elegans*, etc.; human or animal cells, tissues, or biochemical reactions (e.g., ligand binding assays) with in vitro exposure regimens; bioinformatics pathways of disease analysis; or high throughput screening data. These studies are tagged during title and abstract/full-text screening and an iterative approach is used to prioritize for further analysis based on likelihood of the study to impact hazard conclusions or inform toxicity value derivation. Studies that do not undergo further analysis will be classified as PECO-relevant supplemental information.
Exposure	Exposure based on administered dose or concentration, biomonitoring data (e.g., urine, blood, or other specimens), environmental or occupational-setting measures (e.g., air, water levels), or job title or residence. The potential for human exposure to chloroprene primarily is via inhalation and perhaps by the dermal route. ADME and PBPK studies will also be included. Relevant forms are listed below: • Chloroprene (CASRN 126-99-8) or its metabolites, such as (1-chloroethenyl)oxirane or (2-chloro-2-ethenyl)oxirane • Mixture studies will be included if they include a chloroprene-only group (or one of its metabolites)
Comparator	**Human:** A comparison or reference population exposed to lower levels (or no exposure/exposure below detection levels) or to chloroprene for shorter periods of time.
	Animal and in vitro: Quantitative exposure vs. lower or no exposure with concurrent vehicle control group.
Outcome	• All health outcomes (both cancer and noncancer) • ADME and PBPK studies

CASRN = Chemical Abstract Service registry number.

3. METHODS

3.1. LITERATURE SEARCH STRATEGIES

The literature search focused on studies published since completion of the 2010 IRIS Agency Review Draft of the "Toxicological Review of Chloroprene," which covered the literature up through August 2010. The literature search focused only on the chemical name with no limitations on evidence streams (i.e., human, animal, in vitro, in silico) or health outcomes. The databases listed below were searched for the date range of January 1, 2010 through November 3, 2017 using EPA's Health and Environmental Research Online (HERO) database.[1] Full details of the search strategy for each database are presented in Appendix A.

- PubMed (National Library of Medicine)

- Web of Science (Thomson Reuters)

- ToxLine (National Library of Medicine)

3.2. SCREENING PROCESS

Two screeners independently conducted a title and abstract screen of the search results using DistillerSR[2] to identify study records that met the PECO eligibility criteria. In addition to adherence to PECO eligibility criteria, the exclusion criteria noted below were applied.

- Records pertinent to the PECO framework but not containing original data, such as reviews, editorials, or commentaries (the reference lists from these materials, however, are reviewed to identify PECO-relevant studies that may have been missed during database searching).

- Studies that have not been peer reviewed (e.g., conference abstracts, technical reports, theses/dissertations, working papers from research groups or committees, and white papers).

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[1]EPA's HERO database provides access to the scientific literature behind EPA science assessments. The database includes more than 600,000 scientific references and data from the peer-reviewed literature used by EPA to develop its regulations.
[2]DistillerSR is a web-based systematic review software used to screen studies available at
  https://www.evidencepartners.com/products/distillersr-systematic-review-software.

Records that were not excluded based on title and abstract screening advanced to full-text review. Full-text copies of potentially relevant records identified from title and abstract screening were retrieved, stored in the HERO database, and independently assessed by two screeners to confirm eligibility according to the PECO eligibility criteria. At both title/abstract and full-text review levels, screening conflicts were resolved by discussion between the primary screeners with consultation by a third reviewer or technical advisor (if needed) to resolve any remaining disagreements. For citations with no abstract, the articles are initially screened based on all or some of the following: title relevance (title should indicate clear relevance), page numbers (articles two pages in length or less are assumed to be conference reports, editorials, or letters), and PubMed Medical Subject Headings. Assessment of eligibility status of any non-English publications was facilitated by native-language speakers at EPA or Google Translator. During title/abstract or full-text level screening, studies that were not directly relevant to the PECO framework, but could provide supporting information, were categorized (or "tagged") relative to the type of supporting information they provided (e.g., review, commentary, or letter with no original data; exposure only). Conflict resolution is not required during the screening process to identify supporting information (i.e., tagging by a single screener is sufficient to identify the study as potential supportive information).

3.3. STUDY EVALUATION

3.3.1. Epidemiology Studies (Risk of Bias and Sensitivity)

Key concerns for study evaluation were potential *bias* (factors that affect the magnitude and/or direction of an effect) and *insensitivity* (factors that limit the ability of a study to detect a true effect). Bias can result in false positives and negatives, while study sensitivity primarily focuses on the latter. Epidemiology studies were evaluated for bias and study sensitivity in the following domains: exposure measures, outcome measures, participant selection, potential confounding, analysis, selection of reported results, and study sensitivity (see Table 2).

Table 2. Domains of evaluation for epidemiology studies

Domain	Example information
Exposure measures	Source(s) of exposure (consumer products, occupational, an industrial accident) and source(s) of exposure data, blinding to outcome, level of detail for job history data, timing of measurements, type of biomarker(s), assay information, reliability data from repeated-measure studies, validation studies.
Outcome measures	Source of outcome (effect) measure, blinding to exposure status or level, method of measurement/classification, incident vs. prevalent disease, evidence from validation studies, prevalence (or distribution summary statistics for continuous measures).
Participant selection	Study design, timing and location of the study, and who was included? Recruitment process, exclusion and inclusion criteria, type of controls, total participants eligible, comparison between participants and nonparticipants (or followed and not followed), final analysis group. Does the study include potential vulnerable/susceptible groups or life stages?
Potential confounding	Background research on key confounders for specific populations or settings; participant characteristic data, by group; strategy/approach for consideration of potential confounding; strength of associations between exposure and potential confounders and between potential confounders and outcome; degree of exposure to the confounder in the population.
Analysis	Extent (and if applicable, treatment) of missing data for exposure, outcome, and confounders, approach to modeling, classification of exposure and outcome variables (continuous vs. categorical), testing of assumptions, sample size for specific analyses, relevant sensitivity analyses.
Selective reporting	Are results presented with adequate detail for all of the endpoints and exposure measures of interest? Are results presented for the full sample as well as for specified subgroups? Were stratified analyses (effect modification) motivated by a specific hypothesis?
Sensitivity	What are the ages of participants (e.g., not too young in studies of pubertal development)? What is the length of follow-up (for outcomes with long latency periods)? Choice of referent group, the exposure range, and level of exposure contrast between groups is critical (i.e., the extent to which the "unexposed group" is truly unexposed, and the prevalence of exposure in the group designated as "exposed").

The principles and framework for evaluating epidemiology studies are based on the Cochrane Risk of Bias in Nonrandomized Studies of interventions (ROBINS-I) ([Sterne et al., 2016](#)) but modified to address environmental and occupational exposures. The underlying philosophy of ROBINS-I is to describe attributes of an "ideal" study with respect to each of the evaluation domains (e.g., exposure measurement, outcome classification, etc.). Core and prompting questions are used to collect information to guide evaluation of each domain (see Appendix B). Core questions are considered key concepts while prompting questions help the reviewer focus on relevant details under each key domain. In addition, the expected direction of bias is explicitly considered and the impact of a potential bias is incorporated into the study evaluation process. Emphasis is placed on discerning a bias that would be expected to produce a substantive change in the effect estimate. For each study, in each domain question, reviewers reach a consensus on a value of **Good**, **Adequate**, **Poor**, or **Critically Deficient**. These terms are defined as follows:

- A **Good** classification is intended to represent a perfect or close-to-ideal study design and execution.

- An **Adequate** classification represents studies that may have some limitations, but the judgment is made that those limitations are not likely to be severe or to have a substantive impact on the results.

- A **Poor** classification denotes biases or deficiencies that could materially affect the interpretation of the study.

- A **Critically Deficient** classification would represent a flaw that is so serious that the study could not be used.

Emphasis was placed on discerning bias that could substantively change an effect estimate, considering also the expected direction of the bias. Low sensitivity is a bias towards the null. Once the evaluation domains have been classified, these ratings are combined to reach an overall study confidence classification of **High**, **Medium**, **Low**, or **Uninformative**. This classification is based on the classifications in the evaluation domains and will include consideration of the likely impact of the noted deficiencies in bias and sensitivity on the results. Studies with critical deficiencies in any evaluation domain will be classified as **Uninformative**. Other classifications will generally follow a sorting such that **High Confidence** studies would have the highest evaluation ("Good") for all or most domains; **Low Confidence** studies would have a "Poor" evaluation for one or more domains (unless the impact of the particular limitation[s] is judged to be unlikely to be severe), and **Medium Confidence** studies are in between these groups (e.g., most domains receiving a mid-level **Adequate** evaluation, with no limitations judged to be severe). Study evaluation is conducted with at least two reviewers independently assessing each study, with inclusion of a pilot phase to assess and refine the evaluation process, comparison of decisions and reaching consensus among reviewers, and when necessary, resolution of differences by discussion between the reviewers, the chemical assessment team, or technical experts.

3.3.2. Animal Studies (Risk of Bias and Sensitivity)

No animal bioassay studies were identified in the literature search. If present, they would have been evaluated using the animal study quality assessment approach outlined in Appendix C.

3.3.3. Pharmacokinetic (PK)/Physiologically Based Pharmacokinetic (PBPK) Reporting Quality and Applicability

Judgments on the suitability of a model are separated into two categories: scientific and technical (Table 3). The scientific criteria focus on whether the biology, chemistry, and other information available for chemical MOA(s) are justified (i.e., preferably with citations to support use) and represented by the model structure and equations. The scientific criteria are judged based

on information presented in the publication or report that describes the model and do not require evaluation of the computer code. Preliminary technical criteria include availability of the computer code and completeness of parameter listing and documentation. Studies that meet the preliminary scientific and technical criteria proceed to in-depth technical evaluation, which includes a thorough review and testing of the computational code and quality assurance of all parameters and data used in the modeling against original publications, reports, or sources. The in-depth technical and scientific analyses focus on the accurate implementation of the conceptual model in the computational code, use of scientifically supported and biologically consistent parameters in the model, accurate incorporation of parameters and data from their sources, and reproducibility of model results reported in journal publications and other documents. This approach stresses: (1) clarity in the documentation of model purpose, structure, and biological characterization; (2) validation of mathematical descriptions, parameter values, data, and computer implementation; and (3) evaluation of each plausible dose metric. The in-depth analysis is used to evaluate the potential value and cost of developing a new model or substantially revising an existing one.

Table 3. Criteria of evaluation for physiologically based pharmacokinetic (PBPK) models

Criteria	Example information
Scientific	Biological basis for the model is accurate. • Consistent with mechanisms that significantly impact dosimetry. • Predicts dose metrics expected to be relevant. • Applicable for relevant route(s) of exposure.
	Consideration of model fidelity to the biological system strengthens the scientific basis of the assessment relative to standard exposure-based extrapolation (default) approaches. • Can the model describe critical behavior, such as nonlinear kinetics in a relevant dose range, better than the default (i.e., $BW^{3/4}$ scaling)? • Is the available metric a better predictor of risk than default? Specifically, model-based metrics may correlate better than the applied doses with animal/human dose-response data. Degree of certainty in model predictions vs. default is also a factor. For example, while target tissue metrics are generally considered better than blood concentration metrics, lack of data to validate tissue predictions when blood data are available may lead to a choice of the latter.
	Principle of parsimony • Model complexity or biological scale, including number and parameterization of (sub)compartments (e.g., tissue or subcellular levels) should be commensurate with data available to identify parameters.
	Model describes existing PK data reasonably well, both in "shape" (matches curvature, inflection points, peak concentration time, etc.) and quantitatively (e.g., within a factor of 2–3).
	Model equations are consistent with biochemical understanding and biological plausibility.
Initial technical	Well-documented model code is readily available to EPA and public.
	Set of published parameters clearly identified, including origin/derivation.

Criteria	Example information
	Parameters do not vary unpredictably with dose (e.g., any dose dependence in absorption constants is predictable across the dose ranges relevant for animal and human modeling).
	Sensitivity and uncertainty analysis has been conducted for relevant exposure levels (local sensitivity analysis is sufficient, though global provides more information). • If a sensitivity analysis was not conducted, the PKWG would suggest this as additional work before using the model in the risk assessment. • A sound explanation should be provided when sensitivity of the dose metric to model parameters differs from what is reasonably expected based on experience.

BW$^{3/4}$= body-weight scaling to the 3/4 power; PK = pharmacokinetic; PKWG = Pharmacokinetic Working Group

3.4. DATA ABSTRACTION OF STUDY METHODS AND RESULTS

Information on study design and results from epidemiology and animal toxicology studies were extracted into the Health Assessment Workspace Collaborative (HAWC).[3] Key information from identified PK/PBPK models are summarized in tabular format. Data abstraction was performed by one member of the evaluation team and checked by one to two other members. Any discrepancies in data abstraction were resolved by discussion or consultation with a third member of the evaluation team.

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[3]HAWC is a modular, content management system designed to store, display, and synthesize multiple data sources for the purpose of producing human health assessments of chemicals. This online application documents the overall workflow of developing an assessment, from literature search and systematic review, to data extraction (human epidemiology, animal bioassay, and in vitro assay), dose-response analysis, and finally, visualization to facilitate evidence synthesis.

4. RESULTS

4.1. LITERATURE SEARCH RESULTS

The database searches yielded 182 unique records, with no additional records identified from other sources. All studies published after the 2010 IRIS assessment that were cited in the request for correction were identified during database searching. Of the 182 studies identified, 165 were excluded during title and abstract screening, 17 were reviewed at the full-text level, and 9 studies were considered relevant to the PECO eligibility criteria (see Figure 1). Two of the nine studies were considered PECO-relevant "supplemental material" and not further evaluated, leaving seven studies evaluated for impact on 2010 IRIS assessment conclusions (see Table 4).
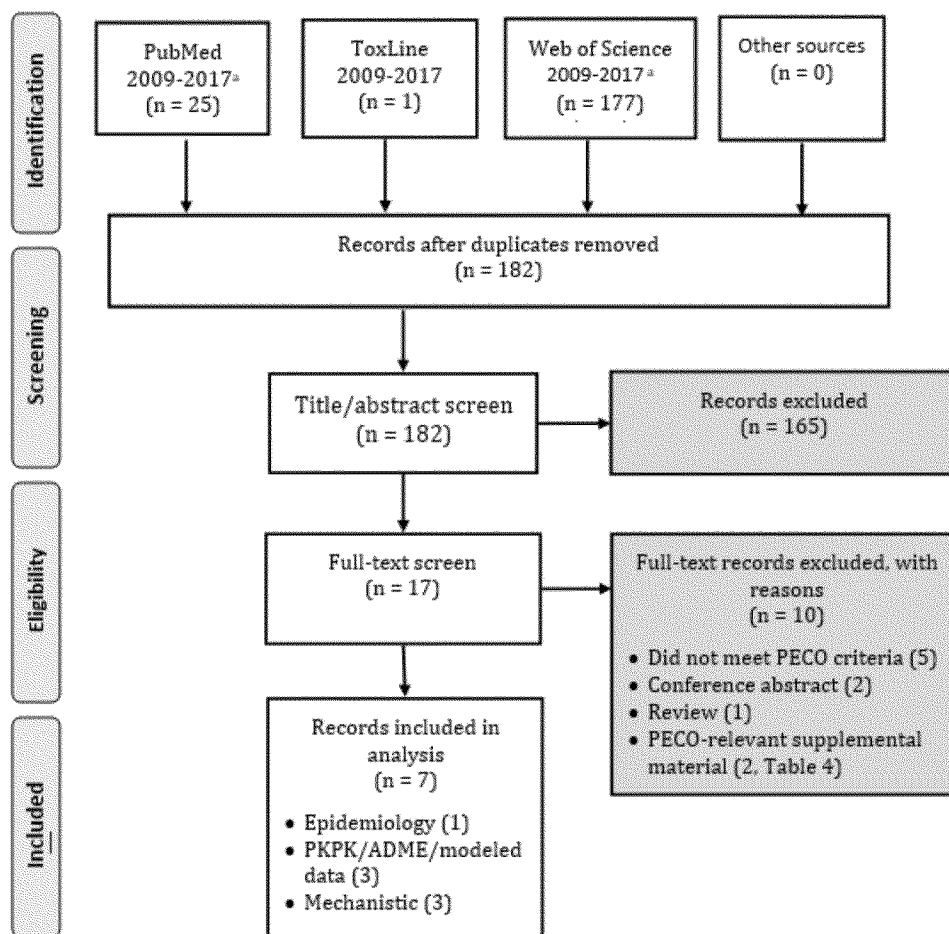
ªJanuary 1, 2010 to November 3, 2017

Figure 1. Study flow selection diagram.

Table 4. Included and population, exposure, comparator, and outcome (PECO)-relevant supplemental material studies

Epidemiology
1. Garcia, E; Hurley, S; Nelson, DO; Hertz, A; Reynolds, P. (2015). Hazardous air pollutants and breast cancer risk in California teachers: a cohort study. Environ Health 14: 14. http://dx.doi.org/10.1186/1476-069X-14-14.

PBPK, ADME, dose-response models
2. Allen, BC; Van Landingham, C; Yang, Y; Youk, AO; Marsh, GM; Esmen, N; Gentry, PR; Clewell, HJ; Himmelstein, MW. (2014). A constrained maximum likelihood approach to evaluate the impact of dose metric on cancer risk assessment: application to β-chloroprene. Regul Toxicol Pharmacol 70: 203-213. http://dx.doi.org/10.1016/j.yrtph.2014.07.001.
3. Eckert, E; Leng, G; Gries, W; Göen, T. (2013). Excretion of mercapturic acids in human urine after occupational exposure to 2-chloroprene. Arch Toxicol 87: 1095-1102. http://dx.doi.org/10.1007/s00204-013-1016-6.
4. Yang, Y; Himmelstein, MW; Clewell, HJ. (2012). Kinetic modeling of β-chloroprene metabolism: Probabilistic in vitro-in vivo extrapolation of metabolism in the lung, liver and kidneys of mice, rats and humans. Toxicol In Vitro 26: 1047-1055. http://dx.doi.org/10.1016/j.tiv.2012.04.004

Mechanistic
5. Guo, Y; Xing, Y. (2016). Weighted gene co-expression network analysis of pneumocytes under exposure to a carcinogenic dose of chloroprene. Life Sci 151: 339-347. http://dx.doi.org/10.1016/j.lfs.2016.02.074.
6. Thomas, RS; Himmelstein, MW; Clewell, HJ; Yang, Y; Healy, E; Black, MB; Andersen, ME. (2013). Cross-species transcriptomic analysis of mouse and rat lung exposed to chloroprene. Toxicol Sci 131: 629-640. http://dx.doi.org/10.1093/toxsci/kfs314.
7. Wadugu, BA; Ng, C; Bartley, BL; Rowe, RJ; Millard, JT. (2010). DNA interstrand cross-linking activity of (1-Chloroethenyl)oxirane, a metabolite of beta-chloroprene. Chem Res Toxicol 23: 235-239. http://dx.doi.org/10.1021/tx9003769.

PECO-relevant supplemental material
8. Gulec, C; Coban, N; Ozsait-Selcuk, B; Sirma-Ekmekci, S; Yildirim, O; Erginel-Unaltuna, N. (2017). Identification of potential target genes of ROR-alpha in THP1 and HUVEC cell lines. Exp Cell Res 353: 6-15. http://dx.doi.org/10.1016/j.yexcr.2017.02.028.
9. Rickert, A; Hartung, B; Kardel, B; Teloh, J; Daldrup, T. (2012). A fatal intoxication by chloroprene. Forensic Sci Int 215: 110-113. http://dx.doi.org/10.1016/j.forsciint.2011.03.029.

4.2. STUDY SUMMARIES AND ANALYSIS

4.2.1. Epidemiology Studies

Garcia, E; Hurley, S; Nelson, DO; Hertz, A; Reynolds, P. (2015). Hazardous air pollutants and breast cancer risk in California teachers: a cohort study. Environ Health 14: 14. *http://dx.doi.org/10.1186/1476-069X-14-14.*

Garcia et al. (2015), in a prospective cohort study of over 112,000 women in California with over 15 years of follow-up, examined the relationship between invasive breast cancer incidence and census tract levels of modeled concentrations of hazardous air pollutants shown to be mammary gland carcinogens. In models assessing the entire cohort, stratifying by age and adjusting for race, an increased risk of breast cancer from exposure to chloroprene was observed among higher quintiles of concentration (Quintiles 4 and 5) as compared to the referent group (Quintiles 1 through 3). Following additional adjustments for multiple comparisons, this relationship did not remain statistically significant. In a sub-group analysis stratifying by age and adjusting for race, a statistically significant association of increased breast cancer risk from exposure to chloroprene (Quintile 5) was found in the BMI $\geq$ 25 subgroup after adjusting for multiple comparisons. Discernable patterns of risk with increasing chloroprene exposure in susceptible population subsets are not clear in this study and may be due to chance. The overall results from this study should be interpreted with caution because exposure estimates were limited to modeled annual average ambient air concentrations from 2002 only and did not account for other exposure sources or routes other than inhalation. The results of this study do not impact the current IRIS hazard conclusions or toxicity values.
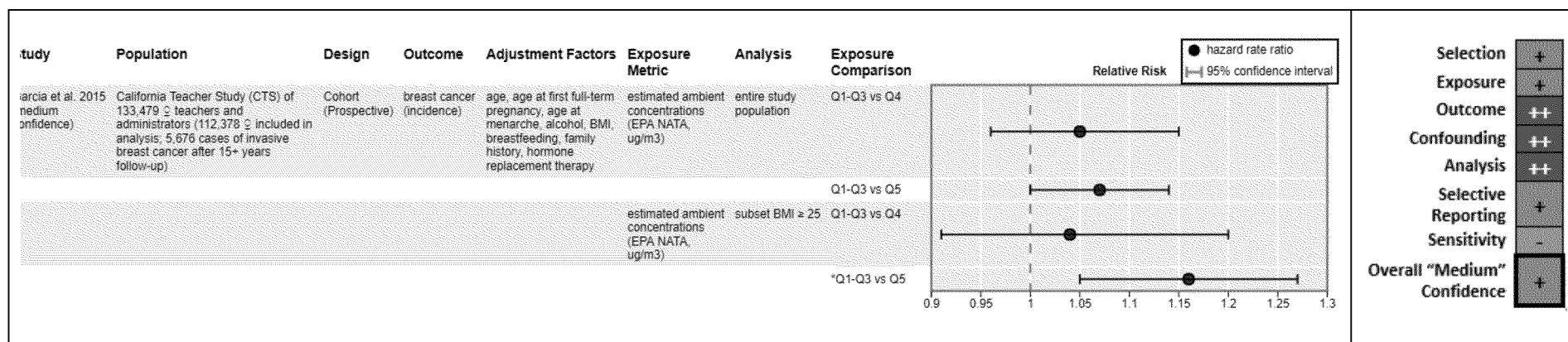
Figure 2. Chloroprene exposure and breast cancer incidence (Garcia et al., 2015).

CTS = California Teacher Study; Q2 = Quintile 2; Q3 = Quintile 3. The study authors collapsed the lower (Q2 and Q3 chloroprene quartiles) into the referent population (Q1) for HR comparison purposes when a larger portion of the study participants had same concentration value; Authors indicated that 71% of women in the CTS had exposure levels of "zero"; the minimum detectable value was ~1E-9 µg/m3 and maximum detectable value was ~1E-2 µg/m3. *The test for trend for chloroprene was statistically elevated at p<0.04. Click to see interactive data graphic and the risk of bias and sensitivity analysis in HAWC.

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Thomas, RS; Himmelstein, MW; Clewell, HJ; Yang, Y; Healy, E; Black, MB; Andersen, ME. (2013). Cross-species transcriptomic analysis of mouse and rat lung exposed to chloroprene. Toxicol Sci 131: 629-640. http://dx.doi.org/10.1093/toxsci/kfs314.

Thomas et al. (2013) conducted a transcriptomic dose-response analysis to identify possible MOAs to explain differences in cross-species lung tumor rates between female B6C3F1/Crl mice and F344/NCrl rats. The animals were exposed for either 5 or 15 days at chloroprene levels of 0.3, 3, 13, or 90 ppm (mice) or 5, 30, 90, or 200 ppm (rats). Following exposure, the animals were sacrificed and their lungs evaluated for histopathology and gene expression via microarray analysis. Following the microarray analysis, a transcriptional benchmark dose (BMD) analysis was conducted on genes shown to be up- or downregulated via gene expression analysis of variance (ANOVA). Histopathology revealed minimal epithelial hyperplasia in most mice exposed to 90 ppm for 5 or 15 days, while no changes were noted in exposed rats. The total number of differentially expressed genes in mice and rats were observed to increase with increasing dose. Differences in gene expression were minimal between mice exposed for 5 or 15 days whereas differences were larger in exposed rats. No genes were differentially expressed at 5 or 30 ppm in rats exposed for 5 days, but rats exposed for 15 days had differentially expressed genes at doses ≥30 ppm. The total number of differentially expressed genes were much larger in rats exposed for 5 versus 15 days. Following transcriptional BMD analysis, the most sensitive pathways in mice were observed to have lower median BMD values (1.12−6.43 ppm) versus those in rats (8.04−29.00). Thomas et al. (2013) observed that induction of Cyp2e1, responsible for the initial oxidation of chloroprene, is similar in the lungs of female rats and mice for exposure levels up to 90 ppm; the mean activity increased by a factor of approximately 1.2- to 1.3-fold, but the change was not statistically significantly different. Cyp2e1 mRNA levels in female rats (exposed to 200 ppm chloroprene for either 5 or 15 days) were increased significantly 1.4-fold over controls; this exposure level was not evaluated in mice, but given the similarity in the trend for mice up to 90 ppm, it appears that mice would have responded similarly to rats at 200 ppm. Conversely, epoxide hydrolase mRNA was induced in mice at >13 ppm (5 or 15 days) and >3 ppm (5 days only), but not rats. Thomas et al. (2013) states "It is not yet known whether the changes in Cyp2e1 and Ephx1 mRNAs are translated into increased enzyme activity, but the ultimate result would be a narrowing of the cross-species differences in the activation-to-detoxification ranges."

The most notable limitation of the Thomas et al. (2013) study for the purpose of evaluating whole-body metabolism is that induction in the kidney and liver and induction in male mice were not evaluated. Thus, the data cannot be used to elucidate the impact of repeated exposure on either whole-body dosimetry or gender differences (or lack thereof) in tumor incidence. Another significant limitation is the length of exposure used. While the limitation of the exposure durations to 5 and 15 days may be useful for identifying affected gene pathways, it remains unclear how these up or down regulations in gene expression relate to possible MOAs of the effects due to chronic exposures to chloroprene as addressed in the 2010 assessment. Also notably missing from the

analysis is any data on humans. While characterizing possible explanations for interspecies differences seen between mice and rats, characterizing differences between mice and humans would have been more informative because mice served as the basis of the cancer analysis to estimate risk in exposed human populations. Thus, the results of this study do not impact the current IRIS toxicity values.

Guo, Y; Xing, Y. (2016). **Weighted gene co-expression network analysis of pneumocytes under exposure to a carcinogenic dose of chloroprene. Life Sci 151: 339-347.** http://dx.doi.org/10.1016/j.lfs.2016.02.074.

Guo and Xing (2016) used the transcriptional data for mice from Thomas et al. (2013) to perform a weighted gene-expression network analysis. Based on the in vivo bioassay results, mice in this study were separated into noncarcinogenic (0.3 and 2 ppm) and carcinogenic (13 and 90 ppm) groups for analysis. The microarray data were normalized and 2,434 genes were identified as being differentially expressed between the two groups; these differentially expressed genes were used to construct a weighted gene coexpression network wherein gene modules and hub genes were identified. A total of 21 gene modules were identified with 12 modules having significantly different gene expression patterns between the noncarcinogenic and carcinogenic groups. For each of these 12 gene modules, a hub gene (genes with high gene significance, module membership, and intramodular interconnectivity) was identified and its possible role in the origin of lung cancer was determined. Hub genes were found to play a role in inflammatory processes (*CFTR*), signaling pathways that can activate *Ras* (*HIP1*), metabolism of chloroprene (*EPHX1*), and control of cell division (*CCND2*). A total of 41 pathways were enriched in the gene modules of interest. Most notably, in the module related to steroid hormone stimulus, the mismatch repair pathway was the most enriched. It is plausible that this pathway is enriched in response to DNA damage induced by exposure to chloroprene. Consensus on approaches to quantitatively integrate these types of genomic results or on how to apply them to replace or even refine risk assessments are not yet currently available. As such, the results of this study do not impact the current IRIS toxicity values.

4.2.2. Physiologically Based Pharmacokinetic (PBPK), Absorption, Distribution, Metabolism, Excretion (ADME), Dose-Response Model

Yang, Y; Himmelstein, MW; Clewell, HJ. (2012). **Kinetic modeling of β-chloroprene metabolism: Probabilistic in vitro-in vivo extrapolation of metabolism in the lung, liver and kidneys of mice, rats and humans. Toxicol In Vitro 26: 1047-1055.** http://dx.doi.org/10.1016/j.tiv.2012.04.004.

Yang et al. (2012) presents the results of the refinement of an existing deterministic PBPK model and the development of a new probabilistic PBPK model (see Table 5). Upon review, there are many apparent concerns about the results presented in this study. These concerns are outlined in Table 6, and are separated into two categories: technical and scientific. These assessments were made based upon the materials available in Yang et al. (2012), and comments submitted to *Docket ID: EPA-HQ-ORD-2009-0217.*

Table 5. Pharmacokinetic (PK)/Physiologically Based Pharmacokinetic (PBPK) model descriptive summary of Yang et al. (2012)

Author	Yang et al. (2012)		
Contact Email	yyang@thehamner.org		
Contact Phone	Tel.: +1 919 558 1310; fax: +1 919 558 1300		
Sponsor	DuPont		
Model Summary			
Species	Mice, rats, humans		
Strain	B6C3F1 mice, F344/*N* rats		
Sex	M/F		
Life-Stage	Adult		
Exposure Routes	Inhalation		
Tissue Dosimetry	Lung	Liver	Kidneys
Model Evaluation			
Language	ACSL 11.8.4		
Code Available:	Sample scripts available in supplemental material. Requests made for full model code. Final in vivo model code should be available.	Effort to recreate model	Significant effort without code
Code Received:	Code for in vitro model received, appears to be complete workspaces; some in vivo model code files received, but they are likely not final. Availability of scripts and in vivo data uncertain.	Migration to new PBPK platform (e.g., R/MCSim)	Unknown effort
Structure Evaluated	Yes		
Math Evaluated	Partially		
Code Evaluated	No		
Available PK Data	Yes (in vitro headspace concentrations)		

F = female; M = male.

Table 6. Technical and scientific evaluation of the Yang et al. (2012) model and analysis

Criteria type and notes	Potential impact on dose-response analysis
Technical (available code): All data and model codes from the Yang et al. (2012) publication are not published or publicly available. PBPK code is necessary for a quality assurance and quality control review by EPA. As a result, EPA cannot evaluate the internal validity of the Yang et al. (2012) PBPK modeling methods or results, or results that are dependent on this model [i.e., Allen et al. (2014)]. Furthermore, code must be translated to a different platform given the discontinuation of acslX software.	Unknown
Scientific (biological basis) and technical (parameters): Female mouse lung metabolism and internal doses in Yang et al. (2012) are not consistent with results for male mice. V_{max} is approximately 5 times higher for male mice than for female mice, yet the tumor response is similar. This has implications for biological basis for the site-specific dose-response, and parameterization of extra-hepatic metabolism (more details provided in subsection below). Also, lung metabolism does not account for tumor responses at other sites, which also need to be incorporated into a risk assessment.	An unknown but major impact due to the importance of the proposed lung internal dose metric. Further evaluation needed if whole-body metabolism is used as a dose metric.
Scientific (model fidelity) and technical (parameters): Female mouse liver and kidney metabolism may be underestimated in Yang et al. (2012). For liver metabolism, this is apparent on the log-scale for predictions of chloroprene headspace concentration data provided in Figure 2b of Yang et al. (2012), and Figures 5 and 25 of Study IISRP-17520-1388 (submitted to EPA-HQ-ORD-2009-0217). The underestimation occurs for both the point estimate results and the Monte Carlo results. Also, because the molecular form of enzymes does not vary between tissues within an individual, or males and females of a species, the K_m for metabolism should be likewise constant across tissues and between sexes.	By mass balance, the error would lead to increased mouse lung metabolism. Increasing mouse internal lung dose would lead to an increased human equivalent concentration if solely applying the lung dose metric (under-estimating human risk). If whole-body metabolism is used to evaluate tumor dose-response in various sites, the impact may be minimal.
Technical (parameters): Possible errors in model optimization for kidney metabolism. Female mouse kidney metabolism approaches zero in MCMC optimization. Parameterization of extra-hepatic metabolism may not be correct (more details provided in subsection below).	

Table 6. Technical and scientific evaluation of the Yang et al. (2012) model and analysis (continued)

Criteria type and notes	Potential impact on dose-response analysis
Technical (MCMC/statistics): likely underestimation of uncertainty, overestimation of significance of differences in parameters between species and sexes: The calculation of likelihood used in the MCMC analysis appears to assume that serially collected samples from each incubation (experimental unit) are treated as independent (i.e., if 20 time points were collected, these are treated as 20 independent samples). But if only a single incubation is conducted, with serial sampling of the headspace, the actual *n* is 1, and the likelihood calculation needs to account for the autocorrelation among repeated measures from a single experimental unit.	Mean parameter values from the MCMC analysis may still be considered sufficient for evaluation of dose-response, but nominal information on the degree of variance or significance of differences between male and female mice, for example, will not be considered. Information from the human microsomal incubations is not sufficient to evaluate interindividual variability.
Technical: model validation vs. in vivo data. The model's ability to reproduce in-vivo PK data [i.e., from Himmelstein et al. (2004a)] has not been evaluated. Of concern is that Himmelstein et al. (2004a) had to reduce alveolar ventilation and total blood flow values predicted from the in vitro data by 50% to match the in vivo PK data presented there. Mice are well known to suppress respiration (RD) and cardiac output in response to irritant gases. However, this response would be dose dependent. A search for RD data for chloroprene in mice was unsuccessful.	Unknown impact on risk predictions. Reductions in ventilation and blood flow needed to match in vivo PK data should assumed to also apply to bioassay conditions, barring data that the response is not chronic. A non-dose-dependent reduction of 50% (i.e., at all exposure levels) may be acceptable. Reduction would only be assumed to occur during periods of exposure.

IISRP = International Institute of Synthetic Rubber Producers; K_m = Michaelis-Menten constant; MCMC = Markov-Chain Monte-Carlo; RD = respiratory depression; V_{max} = maximum expiratory flow.

Other observations regarding Yang et al. (2012) specific to ADME, internal dose, and model/data fitting

Tables 3 and 4 of Yang et al. (2012) report the lung V_{max} to be approximately five times higher for male mice than for female mice. Not surprisingly, the male mouse internal lung dose metric is over fivefold higher than the female mouse at each exposure concentration [Table 5 of Yang et al. (2012)]. However, the tumor profiles between male and female mice are very similar: 26 and 8% (control), 56 and 57% (12.8 ppm), 72 and 68% (32 ppm), and 86 and 84% (80 ppm) (NTP, 1998). Because the fundamental premise of this series of papers is that mouse lung tumors may not be relevant to humans given the large differences in lung metabolism, the reported differences in the internal dose metrics between male mice and female mice should have been explained by the authors. If tumor response can be better explained by using internal dose vs. external concentration, it is unclear how such large differences in metabolism do not translate to differences in tumor incidence. The difference of internal dose between male and female mice is similar to that between female mice and humans [Table 5 of Yang et al. (2012)]. The difference between male and

female mouse internal dose metrics (male/female value) was 5.6-, 5.7-, and 5.4-fold for 12.8, 32, and 80 ppm, respectively. The difference between female mice and humans (female mice/human value) was 7.4, 4.8, and 2.5 at those same doses. The subsequent dose-response analysis by Allen et al. (2014) only incorporates female mouse data, and no rationale for the omission of male mouse data are provided. It cannot be determined whether this discrepancy reflects on the usability or validity of the model because it is possible that site-specific metabolism truly differs substantially between male mice and female mice. However, the discrepancy indicates that the site-specific dose metric may not be appropriate for dose-response modeling and animal-to-human extrapolation.

There are also inconsistencies in the kidney metabolic rates. Anomalies are apparent in the output distributions of the metabolic parameters V_{max} and Michaelis-Menten constant (K_m) for female mice [Figure S6 of Yang et al. (2012) supplementary materials, and Figure 20 of the International Institute of Synthetic Rubber Producers (IISRP)-17520-1388 study]. Unlike for male mice, the probability samples cluster around zero for female mice. The underestimation only occurs for the Monte Carlo results, and the difference between point estimates and Monte Carlo estimates (which are a factor of 10 lower) is attributed only to "background loss rate." It is possible that there was an error in the Markov-Chain Monte-Carlo (MCMC) optimization (i.e., the prior distribution failed to properly incorporate in vitro data, which indicate that kidney metabolism is not zero), and that kidney metabolism is greatly underpredicted in female mice. More reasonable results may have been obtained under the assumption that K_m for Cyp2e1 does not vary between tissues or between males and females (i.e., that only the V_{max} varies between tissues and sexes). To implement this assumption under Bayesian analysis, a hierarchical approach is required to account for the commonality of the K_m within a species. At a minimum, the K_m estimated from the liver data for one sex should be assumed to apply and treated as a fixed constant when evaluating data from the other sex and other tissues.

The model has not been evaluated for its ability to predict in vivo PK data (i.e., there has been no validation of the model). If reductions in respiration rate and cardiac output (total blood flow) are required to match the in vivo data, similar to results of Himmelstein et al. (2004a), then these may be attributed to respiratory depression (RD) which is a response that occurs particularly in mice from exposure to irritant gases. However, such a response would be expected to be dose dependent (lower RD at lower exposure levels). Further, barring data which show that it is not a persistent response, the response should be assumed to also occur during bioassay exposures, but only during periods of exposure.

Other in vivo or in vitro data sets may need to be evaluated further to test model fidelity or validate model parameters. In the chloroprene docket is a report in which blood chloroprene was measured in mice following single (6-hour) and repeated (5- or 15-day) inhalation exposures. Chloroprene blood levels were higher following single exposures, which was postulated to be because of higher minute volume due to stress. The authors conclude that this blood data is suitable for validation of a PBPK model, but it is unclear whether the data were used for the

validation of the PBPK model in <u>Yang et al. (2012)</u>. The report did not investigate chloroprene levels in the organs of interest (namely the lungs, liver, or kidneys).

The metabolic data used to parameterize both the deterministic and probabilistic PBPK models were generated via in vitro headspace experiments where chloroprene was added to closed vials with lung, liver, or kidney microsomal preparations and the disappearance of chloroprene from the vial headspace was measured. Microsomes are derived from the endoplasmic reticulum that contain Phase I and II metabolizing enzymes; microsomes are not present in living cells and are not capable of transcribing mRNA. <u>Thomas et al. (2013)</u> stated that induction of metabolizing enzymes appears to differ between rats and mice, based on data in female rats and mice. However, while Cyp2e1 mRNA levels in female rats (exposed to 200 ppm chloroprene for either 5 or 15 days) were significantly increased over controls, this exposure level was not evaluated in mice. At 90 ppm, female mice and rats had similar levels of Cyp2e1 induction, though not statistically significant vs. controls. Conversely, epoxide hydrolase mRNA was induced in mice at >13 ppm (5 or 15 days) and >3 ppm (5 days only), but not rats. The lack of Cy2e1 induction in the female mouse lung from exposure to 90 ppm chloroprene is supported by an unpublished report submitted to the chloroprene docket (*EPA-HQ-ORD-2009-0217-0009*, report IISRP-12828-1406). This report stated that, "after 15 days of inhalation exposure to β-Chloroprene, no dose-dependent alterations were observed in total CYP content or CYP 1A2, 2B1/2, 2E1, 3A2 or 4A1/2/3 content." <u>Thomas et al. (2013)</u> stated "It is not yet known whether the changes in Cyp2e1 [in rat] and Ephx1 [in mice] mRNAs are translated into increased enzyme activity, but the ultimate result would be a narrowing of the cross-species differences in the activation-to-detoxification ranges." Further evaluation of data is needed to determine the impact (if any) induction would have in humans at environmentally relevant concentrations.

More significantly, data explicitly evaluating metabolic induction in the liver or kidney of female mice or rats, or in any tissue of male mice or rats, are not available. Thus, the possible impact of induction on whole-body metabolism or kinetics in these species, or any difference between males and females, is unknown. PK data submitted to *Docket ID: EPA-HQ-ORD-2009-0217* show a 5.4-fold decrease in chloroprene blood concentration after 15 days of exposure to 13 ppm chloroprene in female mice and approximately 2-fold reductions after 15 days of exposure to 32 and 90 ppm, indicating significant whole-body metabolic induction at these exposure levels. However, if tumor risk is assumed to be proportional to the rate of chloroprene oxidation, the failure to account for this induction in the model is likely to over-estimate the cancer slope factor (i.e., underestimate the dose [rate of metabolism] associated with a particular tumor response). Thus, this inadequacy in the model, under the proposed model application, would result in an error on the side of caution.

Eckert, E; Leng, G; Gries, W; Göen, T. (2013). Excretion of mercapturic acids in human urine after occupational exposure to 2-chloroprene. Arch Toxicol 87: 1095-1102. http://dx.doi.org/10.1007/s00204-013-1016-6. (see Table 7)

Table 7. Absorption, distribution, metabolism, excretion (ADME) inventory/summary of Eckert et al. (2013)

Subjects	14 occupationally exposed individuals (males aged 25–57, median age 43), 30 individuals without occupational exposure (14 males, 16 females, aged 21–63, median age 30). Half of participants in both groups stated as smokers.		
Route	Dermal	Duration	N/A
Analyte(s)	C1-MA-I, C1-MA-III, MHBMA, HOBMA, DHBMA	Matrices	Urine
Exposure	Human biomonitoring pilot study. Significant dermal exposure assumed by the occupational hygienist of the plant. 2-Chloroprene measured in workplace air at <0.1 ppm, and therefore inhalation exposure was assumed negligible.		
Notes	• Elevated levels of the mercapturic acids C1-MA-III, MHBMA, HOBMA, and DHBMA were found in the urine samples of the exposed group. • C1-MA-I and C1-MA-II were not detected in any of the samples. • HOBMA and DHBMA were found in all analyzed urine samples.		

C1-MA-I = 4-chloro-3-oxobutyl MA; C1-MA-II = 4-chloro-3-hydroxybutyl mercapturic acid; C1-MA-III = 3-chloro-2-hydroxy-3-butenyl MA; DHBMA = 3,4-dihydroxybutyl MA; HOBMA = 4-hydroxy-3-oxobutyl MA; MA = mercapturic acid; MHBMA = 2-hydroxy-3-butenyl MA.

Allen, BC; Van Landingham, C; Yang, Y; Youk, AO; Marsh, GM; Esmen, N; Gentry, PR; Clewell, HJ; Himmelstein, MW. (2014). A constrained maximum likelihood approach to evaluate the impact of dose metric on cancer risk assessment: application to β-chloroprene. Regul Toxicol Pharmacol 70: 203-213. http://dx.doi.org/10.1016/j.yrtph.2014.07.001.

The methodology of Allen et al. (2014) has potential for reconciling dose-response relationships from humans and animals when it is not feasible to consider both data types on compatible dose and response scales. However, the reported chloroprene analysis did not use the hazard identification conclusions and dose-response approaches that the 2010 IRIS assessment relied on, so not surprisingly, it estimated a different inhalation unit risk for respiratory cancer than the IRIS assessment. In addition, the use of the PBPK metrics of Yang et al. (2012) for both humans and mice as critical inputs had an unclear impact, owing to the unexplained different rates of chloroprene metabolism in the lung between female and male mice and the unknown impact on projected human internal dose.

The primary difference concerns the human response data for respiratory cancer. The Allen et al. (2014) analysis was based solely on the standardized mortality ratios (SMRs) with external comparison (using U.S. respiratory cancer rates) from the epidemiological study by Marsh et al. (2007). In general, analyses based on internal controls are considered more valid and relevant given concerns including biases such as the healthy worker and healthy worker survivor effects.

Therefore, these SMRs may represent biased estimates, so the slope of zero for the Louisville cohort likely underestimated the magnitude of human responses.

Although there was insufficient support for dose-response estimation, EPA concluded in the 2010 assessment that there was an association of respiratory cancer with increasing chloroprene exposure. The most compelling evidence in the Marsh et al. (2007) paper was the consistent associations, using internal controls, in every upper cumulative exposure quartiles (3 and 4) in the other three plants (odds ratio [OR] range: 1.9–2.9), as well as ORs in excess of 1.0 for low-level exposures in two out of three plants for Quartile 2. Additionally, the cumulative exposure for the Louisville referent group (<4.747 ppm*year) overlapped the exposures in 2nd quartile for the Maydown plant and the 2nd and 3rd quartiles for the Pontchartrain and Grenoble plants. EPA's interpretation of the human evidence was supported by the external peer-review panel; therefore, the choice of the Louisville cohort alone for the Allen et al. (2014) analysis is curious. Given the associations seen in the Maydown, Pontchartrain, and Grenoble cohorts among participants with low exposure levels, the reference choice for the Louisville cohort could attenuate the ability to detect associations at low exposure levels. This would lead to an underestimated slope for the association between chloroprene exposure and lung cancer in that cohort and thus lead to an underestimate of the IUR using the approach of Allen et al. (2014) when combining animal and human data.

Another difference in hazard identification conclusions between the Allen et al. (2014) and the 2010 IRIS assessment concerns multiple tumors observed in mice (and rats), and less sufficient evidence in humans to rule out this possibility. Concerning dose-response approaches, Allen et al. (2014) used a dose-response model that ignored data for decreased time to death with tumor in the mice. Although the human evidence did not support a model including this factor, earlier appearance of tumors was noted in several human studies. Both considerations contributed to a lower potency estimate in mice in the Allen et al. (2014) analysis.

Allen et al. (2014) omitted key information that would clarify applicability of the analysis. First, additional specifics of the dose-response point that both models were constrained to fit would have facilitated a better understanding of the analysis. That is, the cumulative human exposure (either in ppm-years or μmole of metabolite/g lung/day*years) corresponding to the daily PBPK dose of 0.00352 μmole of metabolite/g lung/day was not provided, nor was the response (or range of responses in the uncertainty analysis) estimated at that exposure point.

A second point of needed clarification concerns the final ~1,000-fold range of slope factors, which apparently reflects an uncertainty analysis that only considered the impact of assignments of chloroprene exposures in the Louisville cohort. Without information to clarify what was done, the "maximum-likelihood estimate" within this range then appears to be the slope factor estimate associated with the highest maximum-likelihood combined model fit among all maximum-likelihood estimates from 1,500 different characterizations of the Louisville exposure data. Therefore, both limits of this range, as well as the central tendency estimate, are likely

underestimated by considering only dose-response inputs that minimize estimates of human and animal potency, as opposed to considering the full range of interpretations consistent with the available data. Note: The EPA inhalation unit risk is an upper bound and not directly comparable to a maximum-likelihood estimate.

4.2.3. Carcinogenicity and Mode-of-Action (MOA) Considerations

In their comments on the chloroprene assessment, Ramboll Environ scientists questioned the scientific support for a genotoxic MOA for chloroprene, and instead proposed an alternative MOA involving hyperplasia, induced cell proliferation, and increased expression of pre-existing mutations. The 2010 assessment does not discount the possibility of additional carcinogenic MOAs, and even acknowledges that alternative MOAs may be present at high doses given the decrease in K-ras A to T transversions seen at high doses (i.e., 80 ppm). However, the evidence presented in the 2010 IRIS assessment clearly supports that genotoxicity is a possible MOA. Ramboll Environ scientists note that A to T transversions have been observed in spontaneous mouse lung tumors, but this particular transversion (CAA→CTA at codon 61) was not observed in any historical National Toxicology Program controls, thus decreasing the chance that chloroprene exposure could be increasing the expression of pre-existing mutations. Further, the proposed genotoxic MOA for chloroprene was unanimously supported by the external peer-review committee that reviewed the assessment.

Also, interestingly, most of the studies on which Ramboll Environ scientists cite to support their proposed application of the PBPK model also conclude or report that chloroprene may be operative via a mutagenic MOA. For example, the three Himmelstein toxicokinetic papers all make statements in their introductions regarding the mutagenicity of chloroprene. Himmelstein et al. (2001a) and Himmelstein et al. (2004b) stated that in some tests, but not others, chloroprene appears to be genotoxic. Himmelstein et al. (2004a) stated more strongly that "[t]he mechanistic steps by which CD [β-chloroprene] exposure leads to rodent tumors, while not understood fully, strongly suggest a genotoxic mode of action." Himmelstein et al. (2001b) tested the mutagenicity and clastogenicity of (1-chloroethenyl)oxirane and concluded that "results suggested that CEO [(1-chloroethenyl)oxirane]-induced mutagenicity, but not clastogenicity, may contributed to CD-induced carcinogenicity." The three papers under current consideration (Allen et al., 2014; Thomas et al., 2013; Yang et al., 2012) also made strong statements regarding chloroprene's mutagenicity:

Thomas et al. (2013)—"[t]he current hypothesized mode of action for chloroprene involves bioactivation to a mutagenic metabolite, leading to DNA damage and increased tumors."

Yang et al. (2012)—"[o]ne reactive intermediate formed is the epoxide (1-chloroethenyl)oxirane which was mutagenic in the Ames assay, but not clastogenic at cytotoxic concentrations in vivo. This epoxide also shows reactivity with DNA in vitro and is a potential cross-linking agent."

Allen et al. (2014)—"[t]he initial step in metabolism is oxidation forming a stable epoxide, (1-chloroethenyl)oxirane, a genotoxicant that might be involved in the observed carcinogenicity in animals."

5.CONCLUSIONS

5.1. IMPACT OF NEW LITERATURE ON 2010 INTEGRATED RISK INFORMATION SYSTEM (IRIS) CONCLUSIONS

The seven studies evaluated above represent novel approaches to analyzing existing epidemiologic, toxicological, and toxicokinetic data available for chloroprene. However, as is evident in the discussions of those studies, it is the opinion of the EPA that these studies do not present sufficient evidence or provide adequate rationale for re-evaluating the entire chloroprene toxicity database. Of particular note, there are a number of serious concerns surrounding the development and/or application of the PBPK models (Yang et al., 2012), including poor model optimization of the derived metabolic parameters. A number of issues would need to be addressed in order to update or adapt the Yang et al. (2012) PBPK model for use in revising the chloroprene dose-response assessment. For instance, for the model to be used EPA would need the PBPK code to be replicable on publicly-available software. Due to the discontinuation of the acslX modeling platform, the Yang et al. (2012) model (which includes all model files and scripts) would need to be converted to a different platform. In addition, a revised Yang et al. (2012) model should address the technical and scientific evaluation issues outlined in Table 6, a number of which might substantively impact the dose-response analysis. Finally, the model would need to undergo peer review for it to be considered for potential use in any future assessment of chloroprene health risks.

Thomas et al. (2013) provide only information on gene expression resulting from acute exposures, and likely does not reflect changes in gene expression or MOAs due to chronic exposure, limiting its utility in a chronic human health assessment. Last, the combined dose-response analysis (Allen et al., 2014) relied on judgments that underestimated risk in female mice and particularly underestimated human risk, given existing data. The validity of PBPK model results used by Allen et al. (2014) are also dependent on further evaluations needed for the Yang et al. (2012) model. Collectively, there is low confidence in the published conclusions that human risk of respiratory cancer is up to 100-fold less than that in female mice.

Ultimately, the Agency stands behind the conclusions made in the 2010 IRIS Toxicological Review of Chloroprene, including the derived cancer values. The new studies on chloroprene do not provide a reasonable basis for reassessing the human health effects due to chronic chloroprene exposure.

REFERENCES

Allen, BC; Van Landingham, C; Yang, Y; Youk, AO; Marsh, GM; Esmen, N; Gentry, PR; Clewell, HJ; Himmelstein, MW. (2014). A constrained maximum likelihood approach to evaluate the impact of dose metric on cancer risk assessment: application to β-chloroprene. Regul Toxicol Pharmacol 70: 203-213. http://dx.doi.org/10.1016/j.yrtph.2014.07.001

Eckert, E; Leng, G; Gries, W; Göen, T. (2013). Excretion of mercapturic acids in human urine after occupational exposure to 2-chloroprene. Arch Toxicol 87: 1095-1102. http://dx.doi.org/10.1007/s00204-013-1016-6

Garcia, E; Hurley, S; Nelson, DO; Hertz, A; Reynolds, P. (2015). Hazardous air pollutants and breast cancer risk in California teachers: a cohort study. Environ Health 14: 14. http://dx.doi.org/10.1186/1476-069X-14-14

Gulec, C; Coban, N; Ozsait-Selcuk, B; Sirma-Ekmekci, S; Yildirim, O; Erginel-Unaltuna, N. (2017). Identification of potential target genes of ROR-alpha in THP1 and HUVEC cell lines. Exp Cell Res 353: 6-15. http://dx.doi.org/10.1016/j.yexcr.2017.02.028

Guo, Y; Xing, Y. (2016). Weighted gene co-expression network analysis of pneumocytes under exposure to a carcinogenic dose of chloroprene. Life Sci 151: 339-347. http://dx.doi.org/10.1016/j.lfs.2016.02.074

Himmelstein, MW; Carpenter, SC; Evans, MV; Hinderliter, PM; Kenyon, EM. (2004a). Kinetic modeling of beta-chloroprene metabolism: II. The application of physiologically based modeling for cancer dose response analysis. Toxicol Sci 79: 28-37. http://dx.doi.org/10.1093/toxsci/kfh096

Himmelstein, MW; Carpenter, SC; Hinderliter, PM. (2004b). Kinetic modeling of beta-chloroprene metabolism: I. In vitro rates in liver and lung tissue fractions from mice, rats, hamsters, and humans. Toxicol Sci 79: 18-27. http://dx.doi.org/10.1093/toxsci/kfh092

Himmelstein, MW; Carpenter, SC; Hinderliter, PM; Snow, TA; Valentine, R. (2001a). The metabolism of beta-chloroprene: preliminary in-vitro studies using liver microsomes. Chem Biol Interact 135-136: 267-284. http://dx.doi.org/10.1016/S0009-2797(01)00214-9

Himmelstein, MW; Gladnick, NL; Donner, EM; Snyder, RD; Valentine, R. (2001b). In vitro genotoxicity testing of (1-chloroethenyl)oxirane, a metabolite of beta-chloroprene. Chem Biol Interact 135-136: 703-713. http://dx.doi.org/10.1016/S0009-2797(01)00203-4

Marsh, GM; Youk, AO; Buchanich, JM; Cunningham, M; Esmen, NA; Hall, TA; Phillips, ML. (2007). Mortality patterns among industrial workers exposed to chloroprene and other substances: II. Mortality in relation to exposure. Chem Biol Interact 166: 301-316. http://dx.doi.org/10.1016/j.cbi.2006.08.012

NTP (National Toxicology Program). (1998). Toxicology and carcinogenesis studies of chloroprene (CAS No 126-99-8) in F344/N rats and B6C3F1 mice (inhalation studies) (pp. 1-379). (NTP TR 467; NIH PUB 98-3957). Research Triangle Park, NC. http://ntp.niehs.nih.gov/?objectid=070A80D0-96F2-115A-6354967089C17F9B

Rickert, A; Hartung, B; Kardel, B; Teloh, J; Daldrup, T. (2012). A fatal intoxication by chloroprene. Forensic Sci Int 215: 110-113. http://dx.doi.org/10.1016/j.forsciint.2011.03.029

Sterne, J; Higgins, J; Reeves, B. (2016). ROBINS-I: a tool for assessing risk of bias in non-randomized studies of interventions, Version 7 March 2016 [Computer Program]. Ottawa, Canada: Cochrane Methods Bias. Retrieved from http://www.riskofbias.info

Thomas, RS; Himmelstein, MW; Clewell, HJ; Yang, Y; Healy, E; Black, MB; Andersen, ME. (2013). Cross-species transcriptomic analysis of mouse and rat lung exposed to chloroprene. Toxicol Sci 131: 629-640. http://dx.doi.org/10.1093/toxsci/kfs314

Wadugu, BA; Ng, C; Bartley, BL; Rowe, RJ; Millard, JT. (2010). DNA interstrand cross-linking activity of (1-Chloroethenyl)oxirane, a metabolite of beta-chloroprene. Chem Res Toxicol 23: 235-239. http://dx.doi.org/10.1021/tx9003769

Yang, Y; Himmelstein, MW; Clewell, HJ. (2012). Kinetic modeling of β-chloroprene metabolism: Probabilistic in vitro-in vivo extrapolation of metabolism in the lung, liver and kidneys of mice, rats and humans. Toxicol In Vitro 26: 1047-1055. http://dx.doi.org/10.1016/j.tiv.2012.04.004.

APPENDICES

APPENDIX A. LITERATURE SEARCH STRATEGIES

Table A-1. Literature search strategies

WOS	((TS="Chloroprene" OR TS="1,3-Butadiene, 2-chloro-" OR TS="2-Chloor-1,3-butadieen" OR TS="2-Chlor-1,3-butadien" OR TS="2-Chlorbuta-1,3-dien" OR TS="2-chloro-1,3-butadiene" OR TS="2-Chloro-1,3-butadiène" OR TS="2-chlorobuta-1,3-diene" OR TS="Chloropren") AND PY=(2010–2017))	Results: 157
PUBMED	(("Chloroprene" OR "1,3-Butadiene, 2-chloro-" OR "2-Chloor-1,3-butadieen" OR "2-Chlor-1,3-butadien" OR "2-Chlorbuta-1,3-dien" OR "2-chloro-1,3-butadiene" OR "2-Chloro-1,3-butadiène" OR "2-chlorobuta-1,3-diene" OR "Chloropren") AND ("2010/01/01"[Date - Publication]: "3000"[Date - Publication]))	Results: 24
TOXNET	@AND+@OR+(Chloroprene+"1,3-Butadiene, 2-chloro-"+"2-Chloor-1,3-butadieen"+ "2-Chlor-1,3-butadien"+"2-Chlorbuta-1,3-dien"+"2-chloro-1,3-butadiene"+ "2-Chloro-1,3-butadiène"+"2-chlorobuta-1,3-diene"+"Chloropren"+ @term+@rn+126-99-8)+(@RANGE+yr+2010+2017)+@NOT+@org+pubmed+pubdart+ crisp+tscats	Results: 1

APPENDIX B. CORE AND PROMPTING QUESTIONS TO ASSESS RISK OF BIAS AND SENSITIVITY IN EPIDEMIOLOGY STUDIES

Table B-1. Core and prompting questions to assess risk of bias and sensitivity in epidemiology studies

Core question	Example prompting questions	Example follow-up questions
Exposure Does the exposure measure reliably distinguish between levels of exposure in a time window considered most relevant for a causal effect with respect to the development of the outcome?	For all: • Does the exposure measure capture the major source(s) of variability in exposure among the participants, considering intensity, frequency, and duration of exposure? • Does the exposure measure reflect a relevant time window? If not, can the relationship between measures in this time and the relevant time window be estimated reliably? • Was the exposure measurement likely to be affected by a knowledge of the outcome or by the presence of the outcome (i.e., reverse causality)? For case-control studies of occupational exposures: • Is exposure based on a comprehensive job history describing tasks, setting, time period, and use of specific materials? For biomarkers of exposure, general population: • Is a standard assay used? What are the intra- and interassay coefficients of variation? Is the assay likely to be affected by contamination? Are values less than the limit of detection dealt with adequately? • What exposure time period is reflected by the biomarker? If the half-life is short, what is the correlation between serial measurements of exposure?	Is the degree of exposure misclassification likely to vary by exposure level? If the correlation between exposure measurements is moderate, is there an adequate statistical approach to ameliorate variability in measurements? If there is a concern about the potential for bias, what is the predicted direction or distortion of the bias on the effect estimate (if there is enough information)?

Table B-1. Core and prompting questions to assess risk of bias and sensitivity in epidemiology studies (continued)

Core question	Example prompting questions	Example follow-up questions
Outcome Does the outcome measure reliably distinguish the presence or absence (or degree of severity) of the outcome?	For all: • Is disease ascertainment likely to be affected by knowledge of, or presence of, exposure (e.g., consider access to health care, if based on self-reported history of diagnosis)? For case-control studies: • Is the non-diseased comparison group (e.g., controls in a case-control study) based on objective criteria with little or no likelihood of inclusion of people with the disease? For mortality measures: • How well does cause of death data reflect occurrence of the disease in an individual? How well do mortality data reflect incidence of the disease? For diagnosis of disease measures: • Is diagnosis based on standard clinical criteria? If based on self-report of diagnosis, what is the validity of this measure? For laboratory-based measures (e.g., hormone levels): • Is a standard assay used? Does the assay have an acceptable level of inter-assay variability? Is the sensitivity of the assay appropriate for the outcome measure in this study population?	Is there a concern that any outcome misclassification is non-differential, differential, or both? What is the predicted direction or distortion of the bias on the effect estimate (if there is enough information)?

Table B-1. Core and prompting questions to assess risk of bias and sensitivity in epidemiology studies (continued)

Core question	Example prompting questions	Example follow-up questions
Participant selection Is there evidence that selection into or out of the study (or analysis sample) was jointly related to exposure and to outcome?	For longitudinal cohort: • Did participants volunteer for the cohort based on knowledge of exposure and/or preclinical disease symptoms? Was entry into the cohort or continuation in the cohort related to exposure and outcome? For occupational cohort: • Did entry into the cohort begin with the start of the exposure? • Was follow-up or outcome assessment incomplete and if so, was follow-up related to both exposure and outcome status? • Could exposure produce symptoms that would result in a change in work assignment/work status ("healthy worker survivor effect")? For case-control study: • Were controls representative of population and time periods from which cases were drawn? • Are hospital controls selected from a group whose reason for admission is independent of exposure? • Could recruitment strategies, eligibility criteria, or participation rates result in differential participation relating to both disease and exposure? For population-based survey: • Was recruitment based on advertisement to people with knowledge of exposure, outcome, and hypothesis?	Were differences in participant enrollment and follow-up evaluated to assess bias? If there is a concern about the potential for bias, what is the predicted direction or distortion of the bias on the effect estimate (if there is enough information)? Were appropriate analyses performed to address changing exposures over time in relation to symptoms? Is there a comparison of participants and non-participants to address whether or not differential selection is likely?

Table B-1. Core and prompting questions to assess risk of bias and sensitivity in epidemiology studies (continued)

Core question	Example prompting questions	Example follow-up questions

<u>**Confounding**</u>
Is confounding of the effect of the exposure likely?
	• Is confounding adequately addressed by considerations in…
	
	   a. … participant selection (matching or restriction)?
	
	   b. … accurate information on potential confounders, and statistical adjustment procedures?
	
	   c. … lack of association between confounder and outcome, or confounder and exposure in the study?
	
	   d. … information from other sources?
	
	• Is the assessment of confounders based on a thoughtful review of published literature, potential relationships (e.g., as can be gained through directed acyclic graphing), minimizing potential over-control (e.g., inclusion of a variable on the pathway between exposure and outcome)?
		If there is a concern about the potential for bias, what is the predicted direction or distortion of the bias on the effect estimate (if there is enough information)?

<u>**Analysis**</u> Does the analysis strategy and presentation convey the necessary familiarity with the data and assumptions?	• Are missing outcome, exposure, and covariate data recognized and, if necessary, accounted for in the analysis? • Does the analysis appropriately consider variable distributions and modeling assumptions? • Does the analysis appropriately consider subgroups of interest (e.g., based on variability in exposure level or duration, susceptible subgroups)? • Is an appropriate analysis used for the study design? • Is effect modification considered, based on considerations developed a priori? • Does the study include additional analyses addressing potential biases or limitations (i.e., sensitivity analyses)?	If there is a concern about the potential for bias, what is the predicted direction or distortion of the bias on the effect estimate (if there is enough information)?

APPENDIX C. ASSESSMENT OF RISK OF BIAS AND SENSITIVITY IN ANIMAL STUDIES

Evaluation of animal studies to assess risk of bias and sensitivity was conducted for the following domains: reporting quality, selection or performance bias, confounding/variable control, reporting or attrition bias, exposure methods sensitivity, and outcome measures and results display (see Table C-1).

Table C-1. Domains of evaluation for animal studies

Domain	Metric	Criteria
Reporting quality	Reporting of information necessary for study evaluation	Key information necessary for study evaluation (study would be deemed critically deficient if not reported[a]): • Species, test article description, levels and duration of exposure, endpoints investigated, qualitative or quantitative results. Important information, which should also be reported, is listed below. The brackets contain secondary information that would ideally be reported and, based on the needs of a given assessment, may be considered important, or key, information. • *Test animal*—strain, sex, source (e.g., vendor), husbandry procedures (e.g., housing, feed, mating), [baseline health (e.g., colony monitoring procedures), age or body weight at start of study]. • *Exposure methods*—test article source, description of vehicle control, route of administration, methods of administration (e.g., gavage volume, exposure chamber), [information on stability, purity, analytical verification methods]. • *Experimental design*—periodicity of exposure, animal age/life stage during exposure and at endpoint evaluation(s), [timing of endpoint evaluation(s) (e.g., latency between exposure and testing)]. • *Endpoint evaluations*—procedural details to understand how endpoints were measured; procedural controls, including information on positive and negative controls; [related details (e.g., biological matrix or specific region of tissue/organ evaluated); information on other manipulations (e.g., surgery, co-treatment)]. • *Results presentation*—presents findings for all endpoints of interest that were investigated, information on variability, experimental units assessed, sample size, statistical procedures, (related details, e.g., maternal toxicity in developmental studies, handling of early mortality in long-term bioassays). *Note*: Studies adhering to GLP (good laboratory practices) or to testing guidelines established by (inter)national agencies are assumed to be of good reporting quality.

Table C-1. Domains of evaluation for animal studies (continued)

Domain	Metric	Criteria
Selection or performance bias	Allocation of animals to experimental groups	Ideally, animal studies are randomized, with each animal or litter having an equal chance of being assigned to any experimental group, including controls, and allocation procedures sufficiently described. Less ideally, but generally adequate or good, are studies indicating normalization of experimental groups before exposure, for example according to body weight or litter, but without indication of randomization. The least preferred situation is studies with no indication of how groups were assigned.
Selection or performance bias	Blinding of investigators, particularly during outcome assessment	Good studies will conceal the treatment groups from the researchers conducting the endpoint evaluations (and, in rare but ideal situations, from all research personnel and technicians). Concern regarding blinding may be attenuated when outcome measures are more objective (e.g., as is the case of obtaining organ weights) or measurement is automated using computer-driven systems (e.g., as is the case in many behavioral assessments).
Confounding/variable control	Control for variables across experimental groups	In a good study, outside of the (chemical) exposure of interest, all variables will be controlled for and consistent across experimental groups. Concern regarding additional variables, introduced intentionally or unintentionally, may be mitigated by knowledge or inferences regarding the likelihood and extent to which the variable can influence the endpoint(s) of interest. A very important example to consider is whether the exposure was sufficiently controlled to attribute the effects of exposure to the compound of interest alone. Generally, well-conducted exposures will not have any evidence of coexposures and will include experimental controls that minimize the potential for confounding (e.g., use of a suitable vehicle control). Other examples of variables that may be uncontrolled or inconsistent across experimental groups include protective or toxic factors that could mask or exacerbate effects, diet composition, or surgical procedures (e.g., ovariectomy).
Reporting or attrition bias	Lack of selective data reporting and unaccounted for loss of animals	In a good study, information is reported on all pre-specified outcomes and comparisons for all animals, across treatment groups and scheduled sacrifices. Aspects to consider include whether all study animals were accounted for in the results (if not, are explanations, such as death while on study, and adjustments provided) and whether expected comparisons or certain groups were excluded from the analyses. In some studies, the outcomes evaluated must be inferred (e.g., a suite of standard measures in a guideline study). *Note*: This metric does not address whether quantitative data were reported, nor considers statistical test methods.

Table C-1. Domains of evaluation for animal studies (continued)

Domain	Metric	Criteria
Exposure methods sensitivity	Characterization of the exposure to the compound of interest	Consider whether there are notable issues that raise doubt about the reliability of the exposure levels, or of exposure to the compound of interest. Depending on the chemical being assessed, this may include considering factors such as the stability and composition (e.g., purity, isomeric composition) of the test article, exposure generation and analytic verification methods (including whether the tested levels and spacing between exposure groups is resolvable using current methods), and details of exposure methods (e.g., inhalation chamber type; gavage volume). In some cases, exposure biomarkers in blood, urine, or tissues of treated animals can mitigate concerns regarding inaccurate dosing (dependent on the validity of the biomarker for the chemical of interest). *Note*: While this identifies uncertainties in dose-response, it is typically not a valid reason for exclusion from Hazard ID.
	Use of the exposure design for the endpoint of interest	Based on the known or presumed biological progression of the outcomes being evaluated, consider whether there are notable concerns regarding the timing, frequency, or duration of exposure. For example, better developmental studies will cover a greater proportion of the developmental window thought to be critical to the system of interest, while better studies for assessing cancer or other chronic outcomes will be of longer duration. Studies that expose animals infrequently or sporadically, or, conversely, on a continuous basis (which, depending on the exposure level, can impact food/water consumption, sleep cycles, or pregnancy/maternal care), might introduce additional complications.
Outcomes measures and results display	Sensitivity and specificity of the endpoint evaluations	Consider whether there are notable concerns about aspects of the procedures for, or the timing of, the endpoint evaluations. Based on the endpoint evaluation protocol used for the endpoints of interest, specific considerations will typically include: • Concerns regarding the sensitivity of the specific protocols for evaluating the endpoint of interest (i.e., assays can differ dramatically in terms of their ability to detect effects) and/or their timing (i.e., the age of animals at assessment can be critical to the appropriateness and sensitivity of the evaluation). This includes both overestimates or underestimates of the true effect, as well as a much higher (or lower) probability for detecting the effect(s) being assessed. • Concerns regarding the specificity and validity of the protocols. This includes the use of appropriate protocol controls to rule out nonspecific effects, which can often be inferred from established guidelines or historical assay data. It may be considered useful for insensitive, complex, or novel protocols to include positive and/or negative controls. • Concerns regarding adequate sampling. This includes both the experimental unit (e.g., litter, animal) and endpoint (e.g., number of slides evaluated). This is typically inferred from historical knowledge of the assay or comparable assays. *Notes*: Human relevance of the endpoint is not addressed during study evaluation; for under sampling without blinding (e.g., sampling bias), this will typically lead to gross overestimates of effect; sample size is generally not a reason for exclusion.

Table C-1. Domains of evaluation for animal studies (continued)

Domain	Metric	Criteria
Outcomes measures and results display (continued)	Usability and transparency of the presented data	Consider whether the results are analyzed or presented in a way that limits concerns regarding the reliability of the findings. Items that will typically be important to consider include: • Concern that the level of detail provided does not allow for an informed interpretation of the results (e.g., authors' conclusions without quantitative data; discussing neoplasms without distinguishing between benign and malignant tumors; not presenting variability). • Concern that the way in which the data were analyzed, compared, or presented is inappropriate or misleading. Examples include failing to control for litter effects (e.g., when presenting pup data rather than the preferred litter data), pooling results from males and females or across lesion types, failing to address observed or presumed toxicity (e.g., in assessed animals; in dams) when exposure levels are known or expected to be highly toxic, incomplete presentation of the data (e.g., presenting continuous data as dichotomized), or non-preferred display of results (e.g., using a different readout than is expected for that assay). The evaluator should support how or why, and to what extent, this might mislead interpretations. *Notes*: Concerns regarding the statistical methods applied are not addressed during study evaluation, but should be flagged for review by a statistician. Missing information related to this metric should typically be requested from the study authors.
Other	(Optional)	Example 1: Control for other threats to internal validity. This exceptional metric might be used to consider animal husbandry concerns, reports of predosing toxicity or infection, etc. Example 2: Lack of concern for sensitivity of the animal model. This exceptional metric should be used only when there is demonstrated evidence of differences in model (e.g., species, sex, strain) sensitivity. This does not address the human relevance of the animal model.